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Pesticide and PCB Residues in Northeastern Alberta Otter

Alberta
ENVIRONMENTAL CENTRE

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PESTICIDE AND PCB RESIDUES IN NORTHEASTERN ALBERTA OTTER

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ABSTRACT

The purpose of this study was to measure the concentration of pesticide and PCB residues in otters collected from northeastern Alberta, and to evaluate the potential of otter as an indicator species. Analyses of lipid and liver tissues for 45 different compounds were completed. Determinations encompassed most pesticides commonly used in Alberta now or in the past, and pesticides and PCB's which may originate in Alberta or from outside the province. Based on analyses completed for this project it was concluded that:

1. The chemical residues detected in otter tissues were low indicating that habitats of otter in northeastern Alberta have insignificant chemical residues of pesticides and PCB's.

2. HCB, α-BHC, DDE, DDD, oxy-chlordane, cis-chlordane heptachlor epoxide, dieldrin and PCB's were detected in otter lipid and/or liver. Only HCB, α-BHC, DDE and PCB's were consistently detected. Residues ranged from non-detected (ND) to 2.34 µg/g.

3. Residues of these chemicals should begin to decrease in the future because the use of most chlorinated pesticides and PCB's is decreasing.

4. PCB isomers detected in otter tissues were different than the chromatographic profile routinely produced upon PCB analysis of fish tissues.

5. Monitoring of these compounds should continue elsewhere in Alberta, but emphasis should also focus on heavy metals, and site- specific sampling should continue. Mink may serve a better role as an indicator species than do otter.

PROJECT TEAM

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1. INTRODUCTION

Although there is little that can be done about chemical residues now in the environment, monitoring their occurrence over time can provide a current profile of the chemical residues present and serve as a temporal barometer of environmental quality. The use of organochlorine pesticides such as DDT, dieldrin, aldrin, endrin, and heptachlor for insect control in North America has for the most part been legislatively curtailed during the last decade. Birds of prey, especially the peregrine falcon (Falco peregrinus), were particularly susceptible to persistent, lipophilic, bioaccummulating organochlorine pesticides. Similarily, polychlorinated biphenyls (PCB's) have been widely detected in fish, wildlife and domestic animals because of preferential bioaccumulation of PCB residues in the food chain. PCB's were widely used as hydraulic fluids, plasticizers, flame retardants and as dielectric fluids in capacitors and transformers; however, North American production of PCB's was stopped in the 1970's. Regardless, over 6.4×10^8 kg of PCB's were produced in North America from 1930 - 1975, and thousands of older transformers and capacitors containing PCB's are still in operation. The potential for continued release of PCB's into the environment remains significant.

Regardless of the North American ban on production and use of most organochlorine pesticides and PCB's, monitoring of chemical residues in fish and wildlife indicates that these persistent compounds are still much in evidence throughout North America. Extensive monitoring of pesticides and PCB residues in fish tissues has been carried out world-wide including the Great Lakes Basin of Eastern Canada, and more recently in Alberta (AEC 1983). Monitoring of chemical residues in terrestrial fauna has not in general been as intensive. Data on chemical residue levels in Alberta wildlife, particularly non-migratory species, is for the most part unknown.

Otters (Lutra canadensis) are consumers of fish, invertebrates, amphibians and small mammals co-habiting their aquatic habitat (Gilbert and Nancekivell, 1982). As carnivores at the terminus of their respective food chains, semi-aquatic mammals such as otter and mink (Mustela vison) may be expected to accumulate pesticide, PCB, heavy metal and high molecular weight organic compounds. Otters are not excessively far-ranging compared to birds; thus monitoring of chemical residues in otters should yield a diverse contaminant profile unique to the specific environs from which the animals are collected.Monitoring of mercury in Manitoba otters revealed that because of biomagnification otters accumulated about ten times more mercury than the level detected in fishes from the same aquatic systems (Kucera, 1983). Consequently, even at very low levels of contamination within an ecosystem,

furbearers may serve an indicator role in evaluating chemical residues.

The purpose of this report is to present chemical residue data for otters collected from aquatic habitats in northeastern Alberta. The Alberta Environmental Centre began analysis of tissues in 1981 at the request of Alberta Energy and Natural Resources (Fish and Wildlife Division). The final component of this request was completed in 1984 by completing analysis of tissues from otters collected in the period 1982-83. The initial work was restricted to analysis of pesticide and PCB residues in Alberta otter. Future analysis will include analysis of these same specimens for mercury residues, and also complete analysis of mink tissues for pesticide, PCB and mercury residues.

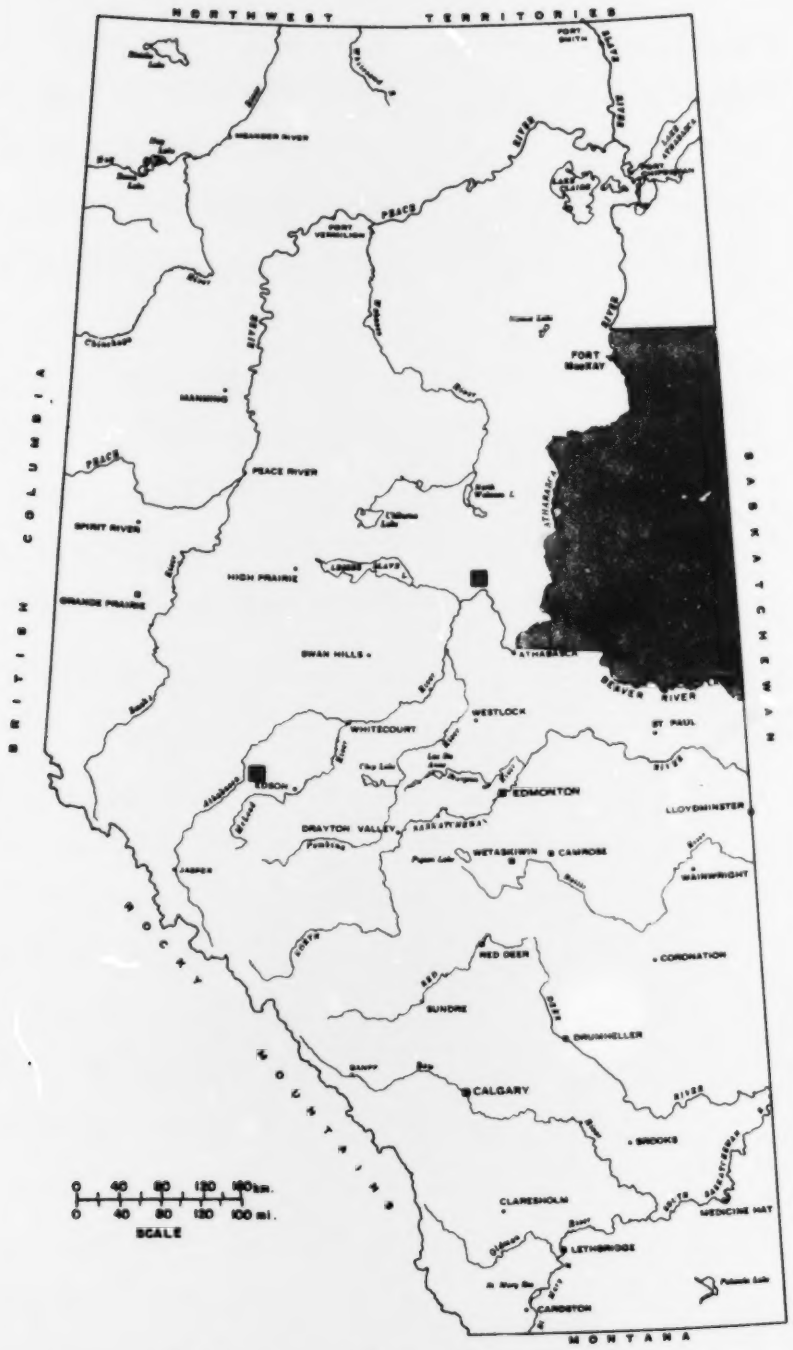


FIGURE I OTTER COLLECTION AREAS

2. METHODS

2.1 Field Collections

Otter carcasses were obtained by Fish and Wildlife personnel from licenced Alberta trappers during the trapping seasons of 1980-81 to 1982-83. The majority of the 158 carcasses submitted came from traplines in northeastern Alberta within an area bounded by the Athabasca River and the Saskatchewan border, and from the Beaver River in the south to about the Fort MacKay latitude in the north (Figure 1). Otter were trapped therefore in both stream and lake associated habitats. Carcasses were kept frozen at -20°C or lower until dissected.

A. Tissue Selection

Fifty-eight (58) otter lipid samples of 1-5 g each, which were removed on dissection of the otter carcasses, were analyzed in 1981. Samples had been wrapped in aluminum foil and stored at -20°C. Sufficient quantities of lipid tissue (abdominal or xiphoid) were not present in some otter carcasses, consequently liver tissues were also selected for residue analysis.

Forty-four (44) liver samples were formed by selecting 12-15 g portions of liver from each of 88 otter and combining such portions to form a pooled sample. Pooling was selected because analysis of individual lipid tissues indicated that residue levels were low,

hence pooling would decrease analysis costs but increase the overall number of carcasses analyzed. Selection of the pooled sub-samples was based on age, sex, location, and storage-age of tissues. Pooled samples of about 25-30 g total were wrapped in aluminum foil and stored at -20°C. Twenty (20) pooled liver samples were formed from carcasses from which lipid tissue had been previously analyzed.

2.2 Chemical Analysis

The chemical methods for determination of pesticide and PCB residues in otter lipid and liver samples were as follows:

A. Lipid Tissues

1. One (1) g of lipid was weighed out and ground in a mortar with 25 g of Florisil containing 5% water and some anhydrous sodium sulfate.

2. A chromatography column was prepared with 25 g of Florisil containing 5% water, then the above sample with Florisil was added on top of it.

3. The petroleum fraction I was obtained by eluting the column with 250 ml of petroleum ether and the fraction II

obtained by a second elution with 275 ml of petroleum ether: methylene chloride (75:25V/V). The "Petroleum Ether Fraction" (Fraction I) contains very non-polar compounds such as HCB, DDE, DDT and PCB's. The Fraction II elution contains slightly polar compounds such as α, β, γ-BHC, Heptachlor Epoxide (HE), Dieldrin, Endrin and DDD.

4. Each fraction was evaporated to near-dryness and reconstituted with a small volume of hexane (eg 5-10 ml) and a small portion (eg 5 ul) of this solution was injected into a gas chromatograph equipped with an electron capture detector. A gas chromatogram thus obtained was compared to the standard pesticide gas chromatogram run along with the sample. The quantities of pesticide in the sample were calculated and expressed in μg/g by comparing the peak height of each pesticide. PCB's were quantified by comparison with Aroclor 1254 or 1260 standard. In most cases Aroclor 1260 PCB isomer standard was used.

B. Liver Tissues

1. The pooled sample (about 25g) of liver was weighed into a 250 ml beaker, and 100 ml of distilled acetonitrile added.

2. The liver was ground in a Polytron grinder and then filtered in vacuum.

3. The filtrate was transferred to a 1-litre separatory funnel with 150 ml of petroleum ether, and 600 ml of petroleum ether-washed distilled water was added. About 5 g of NaCl were added to the separatory funnel and the contents shaken well.

4. The separated petroleum ether layer was dried by passing it through a pad of anhydrous sodium sulfate, and the filtrate evaporated to near-dryness. The moist residue was reconstituted in a minimum amount of petroleum ether and subjected to column chromatography.

5. A chromatography column with 50 g of Florisil containing 5% water was prepared and the concentrate was applied onto the column. Fraction I was eluted with 220 ml of petroleum ether, followed by elution with 275 ml of methylene chloride (75:25 v/v) to obtain Fraction II.

6. Detection and quantification of the pesticide and PCB residues by gas chromatography was the same as for lipid samples.

C. Precision and Accuracy

1. Detection limit (based on 25 g samples):

a) Organochlorine pesticides, 0.001 ppm

b) PCB's, 0.02 ppm (without perchlorination)
0.002 ppm (with perchlorination; Appendix II)

2. Recovery (fortified otter liver):

a) Organochlorine pesticides and PCB's, > 80%

3. Accuracy (according to Gunther, 1970):

Organochlorines/PCB's (ppm)	Accuracy Limits
1.0	± 10%
0.1	± 20%
0.01	± 50%
0.001	±100%

4. Reproducibility (Precision): ±10%

Table 1. List of chemical residues monitored in this study.

HCB (hexachlorobenzene)
α, β, γBHC (hexachlorocyclohexane, or BHC)
Heptachlor
Heptachlor epoxide (HE)
Chlordane (cis)
Oxychlordane
Aldrin
Dieldrin
Endrin
p, p' DDT
p, p'DDE, p, p'DDD
Mirex
Pentachlorobenzene
Toxaphene
Methoxychlor
Aroclor 1242, 1254, 1260 (PCB)
[a]Polybrominated Biphenyls (PBB)

[a]Specific PBB's were not determined; PBB's were determined as a group.

3. RESULTS

Nine of the 22 compounds listed in Table 1 were detected in otter lipid or liver samples. These include: HCB, α-BHC, DDE, DDD, oxy and cis-chlordane, heptachlor epoxide (HE), dieldrin and PCB's. Only HCB, α-BHC, DDE and PCB's were detected in lipid samples (Table 2). The remaining compounds were also detected at very low levels in otter liver samples (Table 2). The presence of low levels of DDD, the chlordanes, HE and dieldrin reflect the catabolic function of the liver enzyme systems in degredation of pesticides compared to the storage function of lipid tissues. Although the concentrations in both tissue matrices were low, the lipophilic properties of HCB, α-BHC, DDE and PCB are evident on comparison of residue levels in lipid and liver tissues (Table 3.) A comparison of lipid and liver chemical residues detected in the same otters presented in Appendices 9 and 10 also illustrates this magnification factor. Liver residue data were calculated on a wet weight basis; not on a lipid concentration basis.

Generally, all compounds ranged from ND (non-detectable) to 0.1 ppm (Appendices 1-4) in otter lipid, and from ND to 0.01 ppm in otter liver (Appendices 5-8). Only PCB residues ranging from ND-2.34 ppm in otter lipid and from ND to about 0.1 ppm in otter liver (Table 2) consistently showed higher levels in the respective tissues. No differences in residue levels between the age or sex-class of otters were evident (Table 3).

TABLE 2. Range in Concentration (µg/g wet weight)[a] of pesticides and PCB's in otter tissues.

Sex	Age[b]	Sample Size	HCB	α-BHC	DDE	DDD	Chlordane Oxy	Chlordane Cis	Heptachlor Epoxide	Diel	PCB
Lipid Tissue											
M	SA	6	0.006-0.097	ND-0.038	ND-0.004	-	-	-	-	-	ND-1.37
	AD	25	0.009-0.038	ND-0.047	ND-0.040	-	-	-	-	-	Tr-1.39
F	SA	11	0.013-0.078	0.004-0.060	ND-0.158	-	-	-	-	-	ND-2.340
	AD	16	0.013-0.064	ND-0.054	ND-0.056	-	-	-	-	-	ND-0.331
Liver Tissue											
M	SA	6	0.001-0.005	Tr-0.001	Tr-0.004	ND	Tr-0.003	ND	ND-0.001	ND-Tr	Tr-0.036
	AD	14	0.001-0.020	Tr-0.001	ND-0.008	ND	Tr-0.008	ND-0.002	Tr-0.001	ND-Tr	Tr-0.047
F	SA	10	0.001-0.004	Tr-0.002	ND-0.006	ND	Tr-0.013	ND-0.006	ND-0.003	ND-0.001	ND-0.084
	AD	14	0.001-0.005	ND-0.002	ND-0.023	ND-0.005	ND-0.003	ND-0.001	ND-0.001	ND	Tr-0.058

[a] ND and -, non-detected (<0.0005 ppm); Tr, trace (<0.001 ppm - 0.0005 ppm>).
[b] SA, sub-adult; AD, adult

TABLE 3. Mean concentration (µg/g wet weight ± SD)[a] of pesticides and PCB's in otter tissues.

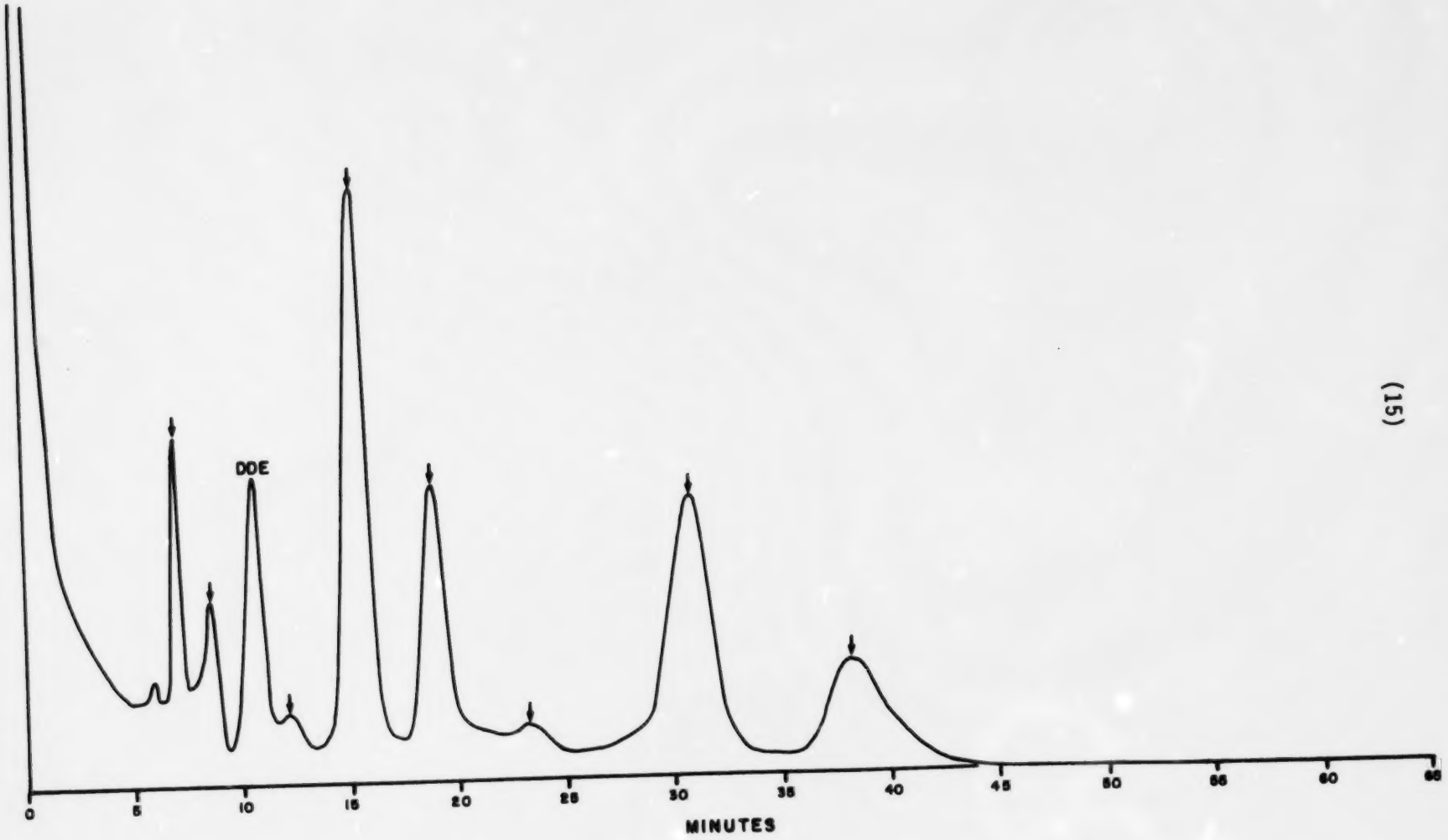
Sex	Age[b]	Sample Size	HCB	α-BHC	DDE	DDD	Chlordane Oxy	Chlordane Cis	Heptachlor Epoxide	Diel	PCB
Lipid Tissue											
M	SA	6	0.036(0.032)	0.012(0.015)	0.001(0.002)	-	-	-	-	-	0.44(0.654)
	AD	25	0.025(0.007)	0.013(0.013)	0.006(0.012)	-	-	-	-	-	0.503(0.438)
F	SA	11	0.031(0.022)	0.032(0.015)	0.020(0.047)	-	-	-	-	-	0.41(0.678)
	AD	16	0.03(0.018)	0.018(0.013)	0.006(0.014)	-	-	-	-	-	0.151(0.122)
Liver Tissue											
M	SA	6	0.003(0.002)	0.001(0.003)	0.002(0.001)	-	0.001(0.001)	-	0.001(0.004)	Tr	0.013(0.014)
	AD	14	0.004(0.005)	Tr	0.002(0.002)	-	0.002(0.002)	Tr	0.001(-)	Tr	0.023(0.014)
F	SA	10	0.002(0.001)	0.001(0.001)	0.002(0.002)	-	0.002(0.004)	Tr	Tr	Tr	0.018(0.025)
	AD	14	0.003(0.001)	Tr	0.003(0.006)	-	0.001(0.001)	Tr	Tr	-	0.012(0.014)

[a] -, non-detected (<0.0005 ppm); Tr, trace (<0.001 ppm - 0.0005 ppm >); SD in parentheses.
[b] SA, sub-adult; AD, adult.

Although lindane (γ-BHC) is still commonly used in Alberta, neither the parent compound nor the β metabolite was detected in the otter tissues sampled. (Appendices 1-8). Small quantities of HE detected in otter liver were perhaps metabolites of chlordane as the use of heptachlor in Canada was banned in 1969. Technical chlordane is a mixture of chlordane, cis (α) and trans (γ) isomers of chlordane, about 10% heptachlor and other compounds (McEwen and Stephensen, 1979). Chromatography also disclosed that the concentration of some PCB isomers in these otter was very low or in many cases specific isomers were totally lacking (Figures 2 and 3). The Aroclor 1260 standard used for identification and quantification of PCB residues in otter and fish tissues (Figure 5) contains 13 definitive PCB isomer peaks in the profile. Fish lipid samples routinely yield 10 - 12 distinct PCB isomers (Figure 4) upon analysis. Otter lipid, however, yielded only 6 major PCB isomer peaks (Figures 2). Lipid extracted from otter muscle tissue also disclosed only 6 PCB isomers.

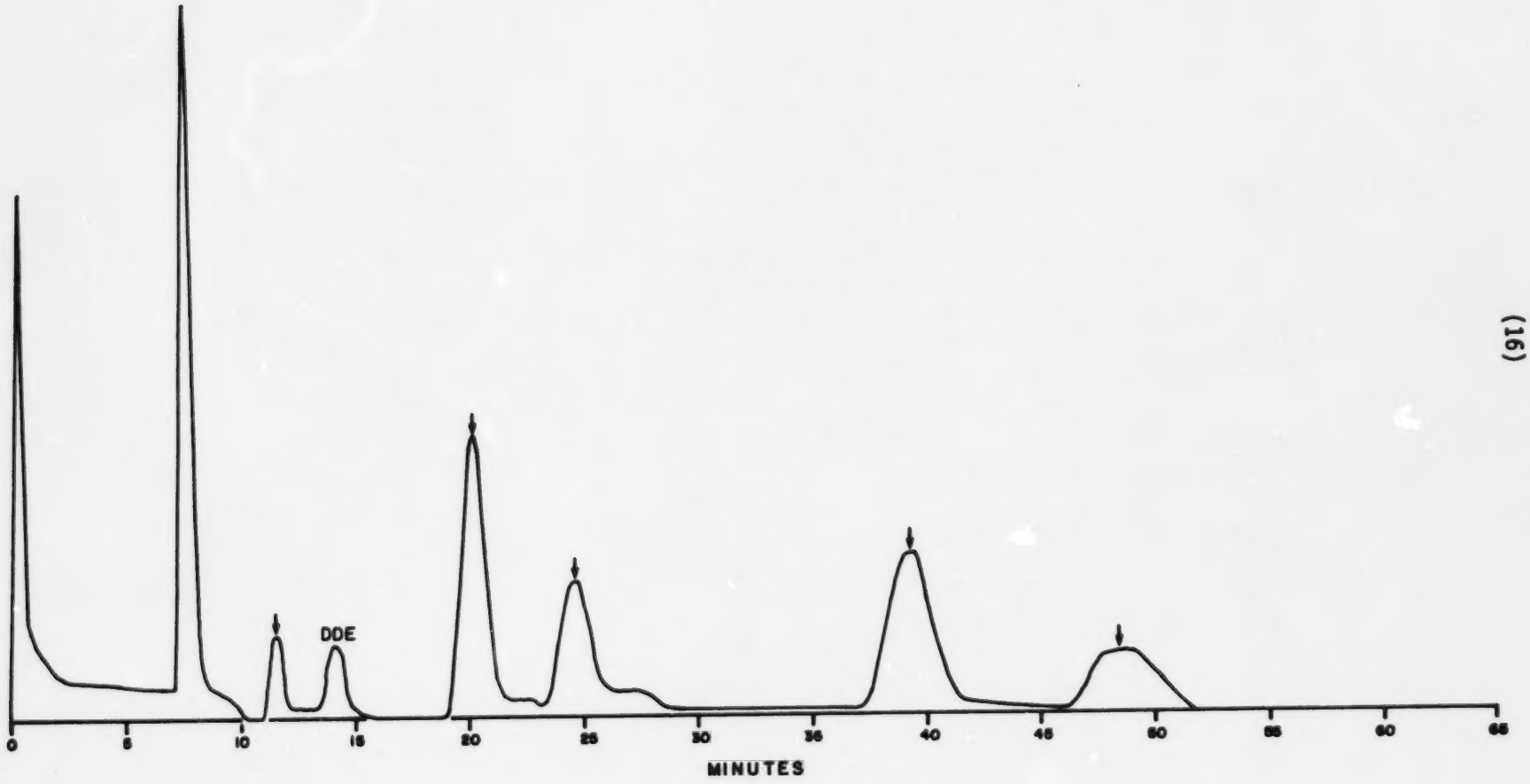
4. DISCUSSION

Overall, the organochlorine pesticide and PCB residues detected in otter tissues were generally low and probably biologically insignificant. This is not surprising in that the use



(15)

FIGURE 2. CHROMATOGRAM OF PCB ISOMERS (↓) DETECTED IN OTTER LIPID



(16)

FIGURE 3. CHROMATOGRAM OF PCB ISOMERS (↓) DETECTED IN OTTER LIVER

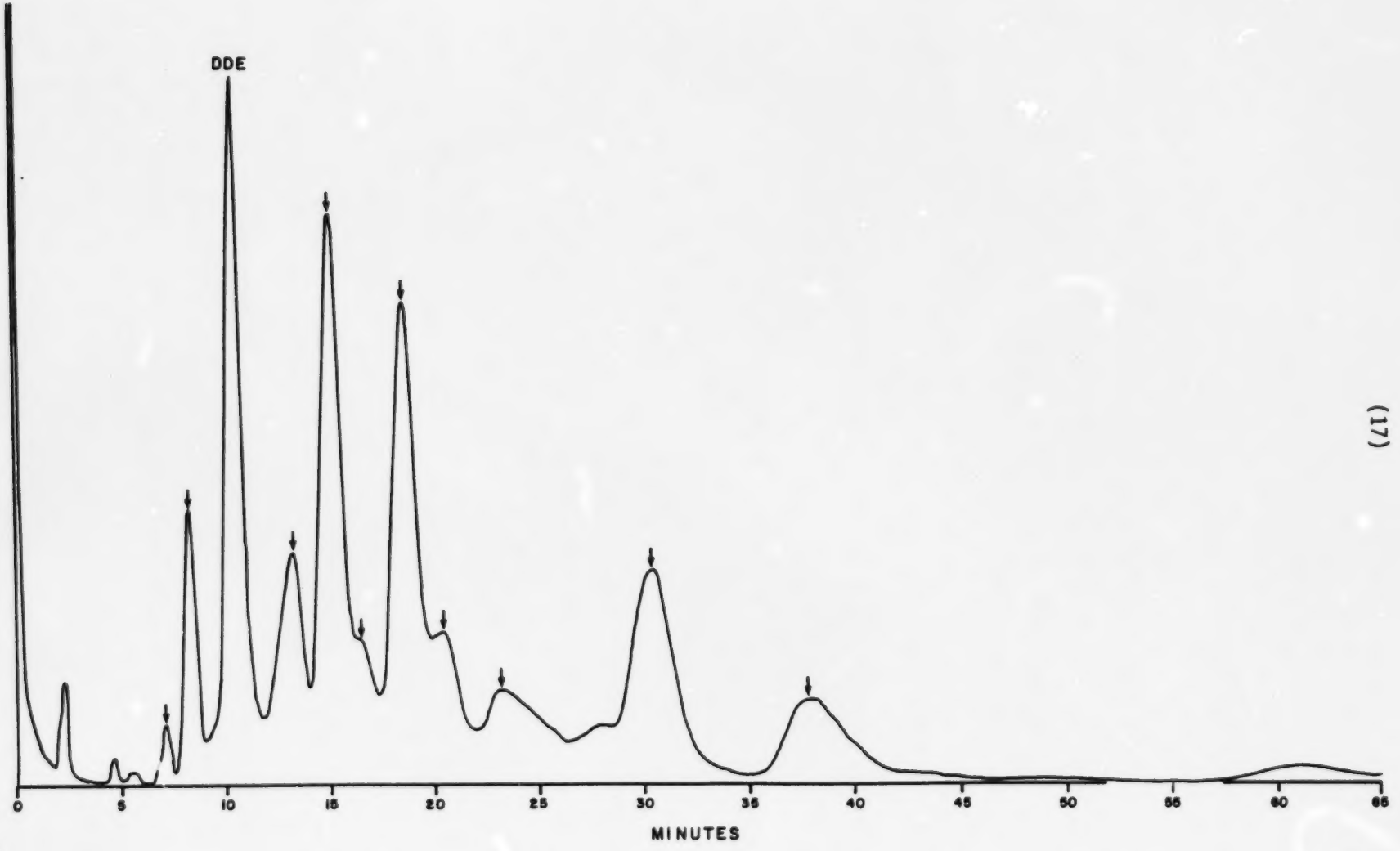


FIGURE 4. CHROMATOGRAM OF PCB ISOMERS (↓) DETECTED IN GOLDEYE LIPID

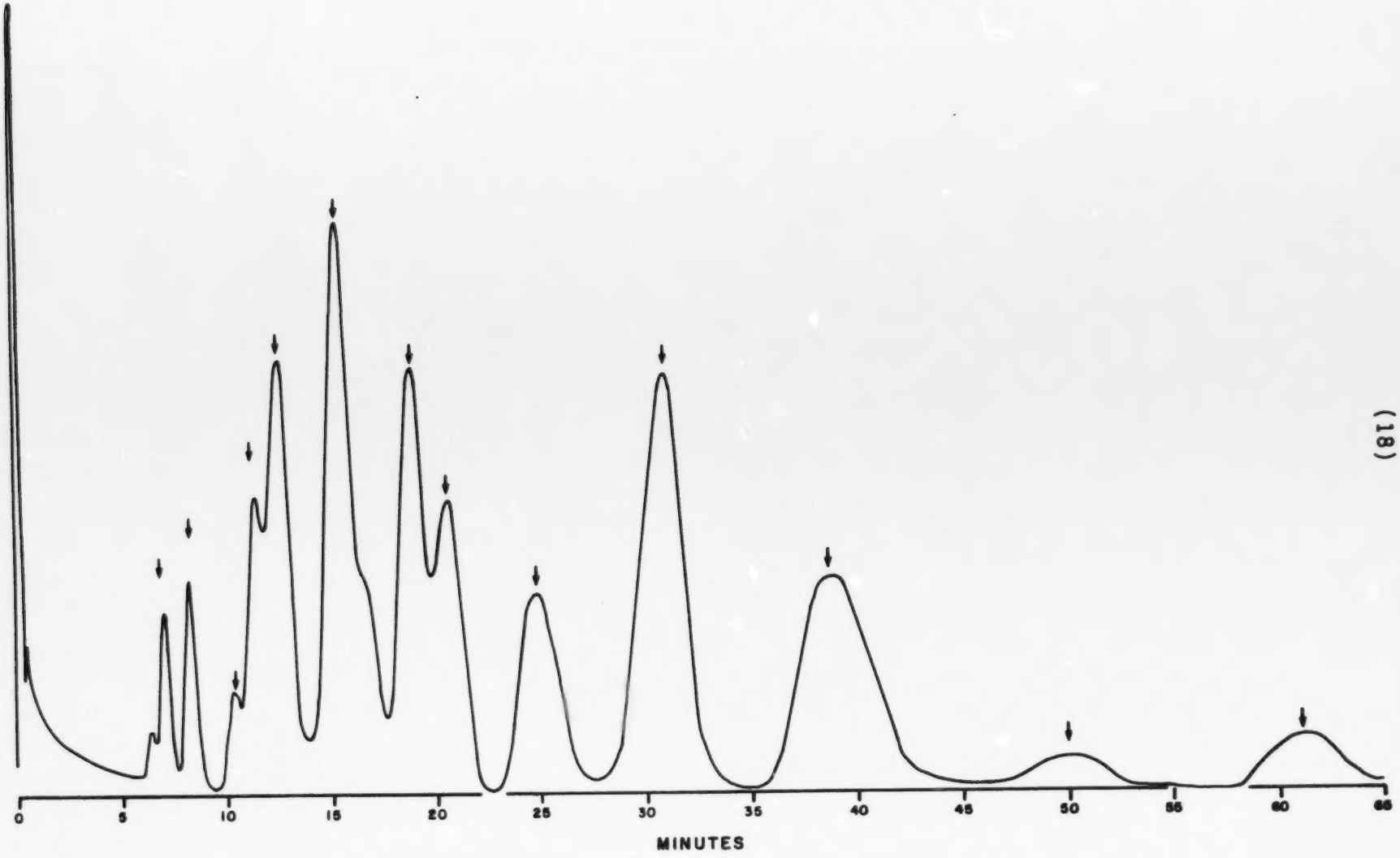


FIGURE 5. CHROMATOGRAM OF AROCLOR 1260 STANDARD

of organochlorine pesticides for agricultural purposes would be low in northeastern Alberta compared to more intensely farmed and populated parts of the country. In addition, very few organochlorine pesticides are still in use in Alberta, Canada or the rest of North America. Lindane (γ-BHC) is the only organochlorine insecticide that is still widely used in Alberta. About 52,000 kg of lindane were used in Alberta as a seed treatment or for the control of ecto-parasites of cattle in 1980. Again this direct source of lindane in the food-chain of otters in northeastern Alberta is unlikely. Regardless of the source, the levels of α-BHC recovered from otter tissues were of no consequence. Lindane deposited in body lipids is metabolized to much less toxic water soluble products which are excreted in the urine. Furthermore, lindane is relatively more volatile and more water soluble than other organochlorine compounds, and the γ-BHC isomer can be isomerized to the more stable α-and β-isomers. Thus, γ and β isomers as in this study are seldom found in animal tissues. The α-BHC metabolite of lindane detected is most likely resultant of atmospheric fallout (Eisenreich et al., 1981), rather than the use of lindane as a pesticide in northeastern Alberta.

Similarily, the HCB (hexachlorobenzene) residues detected in otter tissues are perhaps from air/precipitation-borne depositions to the habitats and food-chains of these northeastern Alberta otter. HCB is a petrochemical waste product, a contaminant in some

pesticides, a fungicide, and also an intermediate breakdown product in lindane degradation. HCB is no longer registered as a fungicide in Canada; however, HCB is a persistent and bioaccumulating compound which is now recognized as a global pollutant. Although HCB can cause disorders in porphyrin metabolism of many species (Courtney, 1979), the overall HCB residue level of about 0.03 µg/g detected in otter lipid would be of no toxicological significance.

This same interpretation would apply to the significance of PCB residues reported herein. Atmospheric transport and the long half-life of PCB's will in all likelihood yield PCB residues in chemical residue profiles for many years to come. Although low in concentration and infrequent in occurrence, the presence of DDE residues in otter tissues many years after DDT use was banned suggests that PCB's will also require a long period for ecosystems to be free of this contaminant. Because the use of PCB's has been curtailed in Alberta, residues should slowly decrease to even lower levels in the future; barring industrial spillage.

Average PCB levels of about 0.4 µg/g as detected in these otter lipid samples would not influence the health or reproduction of the northeastern Alberta otter sampled in this study. Although PCB tissue residues were not reported, Aulerich and Ringer (1977) found that the health and reproduction of mink was not impaired during an 8-month controlled feeding trial until dietary PCB levels exceeded 2 µg/g. Jensen et al. (1977) found that lipid residues of 14 µg/g PCB had no effect on reproduction of female mink. Mobilization and metabolism of the small amounts of PCB's detected

in this study would therefore have little effect, if any, on these northeastern Alberta otter. The concentration and persistence of PCB's is important; however, the chemical composition of PCB isomers is perhaps more important for evaluating the significance and toxicity of PCB residues.

Generally, highly chlorinated PCB homologs are more persistent and more toxic than lower chlorinated biphenyls; however, the position of the chlorine atoms on the PCB isomer also influence toxicity and persistence. For example, 2, 3, 6, 2′, 3′, 6′ hexachlorobiphenyl is readily metabolized and excreted by rats, while the 2, 4, 5, 2′, 4′, 5′ PCB isomer is extremely persistent and very little is metabolized by rats (Birnbaum, 1983). PCB's are primarily metabolized by mammals and birds via hydroxylation with the formation of an arene oxide intermediary; with preferential elimination of the lower chlorinated isomers. Subsequently, mono and dihydroxy PCB metabolites are excreted in the urine and feces. Hydroxylation usually proceeds without dechlorination; however, dechlorination and isomerization, again via an arene oxide intermediate, appears to be a route for metabolism of the more highly chlorinated biphenyls (Safe, 1984).

Although PCB-metabolizing capability exists in fish, the activity of PCB metabolism appears to be relatively low (Safe, 1984). Regardless, the lack of specific PCB isomers in otter tissues reported in this study, compared to fish lipid/muscle PCB residues, suggests that fish-eating furbearers may be consuming potentially toxic PCB isomers or related compounds (eg.

dibenzofurans) that fish are unable to, or slow at transforming to a less toxic state. The type of PCB's in the food chain of these otters may also have been different than that of fish monitored elsewhere in Alberta by the AEC. Only isomer-specific PCB analysis or GC/MS analysis would identify differences in PCB isomers between fish and otter.

Mink are particularly sensitive to the toxic effects of PCB's (Aulerich et al., 1971); thus monitoring of organochlorine compounds in this species, with emphasis on evaluating PCB isomers, is desirable. Mink are more wide-spread throughout Alberta than are otter, most of their food comes from water, and mink range over smaller areas than do otter, consequently mink may be more suitable than otter for evaluating chemical residues in aquatic ecosystems. Mink will be particularly suitable for monitoring in southern Alberta ecosystems where otter are not common.

Future emphasis should be placed on monitoring of heavy metals in fur-bearers, because PCB exposure can synergistically increase the accummulation of cadmium in mink and possibly other heavy metals (Olson et al., 1979). Monitoring of fur-bearers should therefore focus on mink from southern environs where the agricultural and industrial base is more developed than in northeastern Alberta.

REFERENCES

Alberta Environmental Centre, 1983. Chemical residues in fish tissues in Alberta III. Pesticides and PCB's in the North Saskatchewan River and Red Deer River. AECV83-R4. 47 pp.

Aulerich, R.J. and R.K. Ringer. 1977. Current status of PCB toxicity to mink, and effect on their reproduction. Arch. Environ. Cont. Toxicol. 6:279-292.

Aulerich, R.J., R.K. Ringer, H.L. Seagram, and W.G. Youatt. 1971. Effect of feeding coho salmon and other Great Lakes fish on mink reproduction. Can. J. Zool. 49: 611-616.

Birnbaum, L.S. 1983. Distribution and excretion of 2, 3, 6, 2′, 3′, 6′- and 2, 4, 5, 2′, 4′, 5′- hexachlorobiphenyl in senescent rats.

Courtney, K.D. 1979. Hexachlorobenzene (HCB): A Review. Environ. Res. 20: 225-266.

Eisenreich, S.J., B.B. Looney, and J.D. Thornton. 1981. Airborne organic contaminates in the Great Lakes ecosystem. Environ. Sci. and Technol. 15: 30-38.

Gilbert, F.F., and E.G. Nancekivell. 1982. Food habits of mink (Mustela vison) and otter (Lutra canadensis) in north-eastern Alberta. Can. J. Zool. 60: 1282-1288.

Gunther, F.A. 1970. Pesticide residues in the total environment: reliable detection and determination, mitigation, and legislative control and surveillance programs. Pure and Applied Chemistry 21: 235.

Jensen, S., J.E. Kihlstrom, M. Olsson, C. Lundberg and J. Orberg. 1977. Effects of PCB and DDT on mink (Mustela vision) during the reproductive season. Ambio. 6:239.

Kucera, E. 1983. Mink and otter as indicators of mercury in Manitoba waters. Can. J. Zool. 61: 2250-2256.

McEwen, F.L., and G.R. Stephenson. 1979. The use and significance of pesticides in the environment. John Wiley and Sons Inc., Toronto, Ont. 538 pp.

Olsson, M., J.E. Kihlstrom, S. Jensen, and J. Orberg. 1979. Cadmium and mercury concentrations in mink (Mustela vison) after exposure to PCB's. Ambio. p. 25.

Safe, S. 1984. Polychlorinated biphenyls (PCB's) and polybrominated biphenyls (PBB's): Biochemistry, toxicology, and mechanism of action. CRC Critical Rev. in Toxicol. 13: 319-395.

Appendix 1. Pesticide and PCB residues (µg/g wet weight)[a] in sub-adult male otter lipid.

Sample #	HCB	αBHC	Lind	DDE	DDD	Chlordane Oxy	Chlordane Cis	Heptachlor Epoxide	Diel	PCB
1	0.018	-	-	-	-	-	-	-	-	Tr
2	0.032	0.004	-	-	-	-	-	-	-	-
3	0.036	0.018	-	0.004	-	-	-	-	-	1.19
4	0.025	0.013	-	-	-	-	-	-	-	-
5	0.097	0.038	-	-	-	-	-	-	-	1.37
6	0.006	-	-	-	-	-	-	-	-	0.077

[a]-, non-detected (<0.0005 ppm); Tr, trace (<0.001 ppm - 0.0005 ppm>).

Appendix 2. Pesticide and PCB residues (µg/g wet weight)[a] in adult male otter lipid.

Sample #	HCB	αBHC	Lind	DDE	DDD	Chlordane Oxy	Chlordane Cis	Heptachlor Epoxide	Diel	PCB
1	0.030	0.009	-	-	-	-	-	-	-	0.715
2	0.038	0.022	-	-	-	-	-	-	-	0.136
3	0.034	0.005	-	-	-	-	-	-	-	0.578
4	0.029	0.009	-	-	-	-	-	-	-	Tr
5	0.016	0.015	-	-	-	-	-	-	-	0.348
6	0.019	0.006	-	-	-	-	-	-	-	0.837
7	0.029	0.044	-	0.040	-	-	-	-	-	1.270
8	0.032	0.047	-	0.040	-	-	-	-	-	1.390
9	0.025	0.016	-	-	-	-	-	-	-	0.368
10	0.029	0.016	-	-	-	-	-	-	-	1.220
11	0.024	-	-	-	-	-	-	-	-	0.121
12	0.025	0.017	-	-	-	-	-	-	-	0.098
13	0.024	-	-	-	-	-	-	-	-	0.403
14	0.016	-	-	-	-	-	-	-	-	Tr
15	0.021	-	-	-	-	-	-	-	-	0.421
16	0.021	-	-	-	-	-	-	-	-	0.984
17	0.009	-	-	-	-	-	-	-	-	0.514
18	0.014	-	-	-	-	-	-	-	-	1.010
19	0.035	0.014	-	0.020	-	-	-	-	-	0.898
20	0.028	0.013	-	0.016	-	-	-	-	-	0.709
21	0.020	0.007	-	-	-	-	-	-	-	0.053
22	0.021	0.001	-	-	-	-	-	-	-	0.067
23	0.029	0.016	-	0.012	-	-	-	-	-	0.383
24	0.034	0.028	-	0.019	-	-	-	-	-	0.028
25	0.018	0.030	-	-	-	-	-	-	-	0.034

[a] -, non-detected (<0.0005 ppm); Tr, trace (<0.001 ppm -0.0005 ppm>).

Appendix 3. Pesticide and PCB residues (µg/g wet weight)[a] in sub-adult female otter lipid.

Sample #	HCB	αBHC	Lind	DDE	DDD	Chlordane Oxy	Chlordane Cis	Heptachlor Epoxide	Diel	PCB
1	0.028	0.035	-	-	-	-	-	-	-	-
2	0.027	0.004	-	0.027	-	-	-	-	-	0.340
3	0.078	0.041	-	0.032	-	-	-	-	-	0.677
4	0.027	0.023	-	0.158	-	-	-	-	-	2.340
5	0.016	0.020	-	-	-	-	-	-	-	0.480
6	0.013	0.018	-	-	-	-	-	-	-	0.145
7	0.015	0.044	-	-	-	-	-	-	-	0.219
8	0.068	0.060	-	0.005	-	-	-	-	-	0.309
9	0.015	0.028	-	-	-	-	-	-	-	-
10	0.019	0.039	-	-	-	-	-	-	-	-
11	0.021	0.040	-	-	-	-	-	-	-	-

[a] -, non-detected (<0.0005 ppm).

Appendix 4. Pesticide and PCB residues (µg/g wet weight)[a] in adult female otter lipid.

Sample #	HCB	αBHC	Lind	DDE	DDD	Chlordane Oxy	Chlordane Cis	Heptachlor Epoxide	Diel	PCB
1	0.022	0.015	-	-	-	-	-	-	-	-
2	0.020	0.018	-	-	-	-	-	-	-	-
3	0.035	0.013	-	-	-	-	-	-	-	-
4	0.030	0.007	-	-	-	-	-	-	-	0.087
5	0.017	0.016	-	-	-	-	-	-	-	0.252
6	0.013	0.011	-	-	-	-	-	-	-	0.014
7	0.017	0.014	-	-	-	-	-	-	-	0.200
8	0.013	0.024	-	-	-	-	-	-	-	0.275
9	0.019	0.010	-	-	-	-	-	-	-	0.194
10	0.064	0.054	-	0.056	-	-	-	-	-	0.331
11	0.060	0.013	-	0.008	-	-	-	-	-	0.115
12	0.048	0.020	-	0.012	-	-	-	-	-	0.324
13	0.058	0.015	-	0.013	-	-	-	-	-	0.255
14	0.016	-	-	-	-	-	-	-	-	Tr
15	0.013	0.026	-	-	-	-	-	-	-	0.140
16	0.017	0.038	-	-	-	-	-	-	-	0.228

[a] -, non-detected (<0.0005 ppm); Tr, trace (<0.001 ppm - 0.0005 ppm>)

Appendix 5. Pesticide and PCB residues (µg/g wet weight)[a] in sub-adult male otter liver.

Sample #	HCB	αBHC	Lind	DDE	DDD	Chlordane Oxy	Chlordane Cis	Heptachlor Epoxide	Diel	PCB
1	0.002	0.001	-	0.001	-	0.001	-	Tr	Tr	0.017
2	0.001	Tr	-	Tr	-	0.001	-	-	-	Tr
3	0.004	Tr	-	0.002	-	0.001	-	Tr	Tr	0.005
4	0.005	0.001	-	0.002	-	0.003	-	0.001	Tr	0.016
5	0.005	Tr	-	0.001	-	0.002	-	0.001	Tr	0.036
6	0.002	0.001	-	0.004	-	Tr	-	Tr	-	Tr

[a]-, non-detected (< 0.0005 ppm); Tr, trace (<0.001 ppm - 0.0005 ppm>).

Appendix 6. Pesticide and PCB residues (µg/g wet weight)[a] in adult male otter liver.

Sample #	HCB	αBHC	Lind	DDE	DDD	Chlordane		Heptachlor Epoxide	Diel	PCB
						Oxy	Cis			
1	0.002	Tr	-	Tr	-	Tr	-	Tr	-	0.027
2	0.007	0.001	-	0.006	-	0.003	0.001	0.001	Tr	0.047
3	0.006	Tr	-	0.001	-	0.004	-	0.001	Tr	0.029
4	0.020	Tr	-	0.008	-	0.008	0.002	0.001	Tr	0.033
5	0.001	Tr	-	0.002	-	0.001	-	0.001	-	0.012
6	0.002	Tr	-	0.002	-	0.001	-	Tr	-	0.014
7	0.001	Tr	-	-	-	0.001	-	Tr	-	0.007
8	0.001	Tr	-	Tr	-	0.001	-	Tr	-	0.005
9	0.002	Tr	-	-	-	0.001	-	0.001	-	0.026
10	0.002	Tr	-	Tr	-	0.001	-	Tr	-	0.024
11	0.002	Tr	-	-	-	0.001	-	Tr	-	0.046
12	0.002	Tr	-	-	-	Tr	-	Tr	-	Tr
13	0.003	Tr	-	Tr	-	0.001	-	Tr	-	0.032
14	0.002	Tr	-	Tr	-	0.001	-	Tr	-	0.021

[a] -, non-detected (<0.0005 ppm); Tr, trace (<0.001 ppm - 0.0005 ppm>)

Appendix 7. Pesticide and PCB residues (µg/g wet weight)[a] in sub-adult female otter liver.

Sample #	HCB	αBHC	Lind	DDE	DDD	Chlordane Oxy	Chlordane Cis	Heptachlor Epoxide	Diel	PCB
1	0.004	0.001	-	0.003	-	0.001	-	-	-	0.031
2	0.002	0.001	-	0.001	-	Tr	-	-	-	0.006
3	0.004	0.001	-	0.006	-	0.002	-	0.001	-	0.016
4	0.003	Tr	-	0.002	-	0.001	-	0.002	Tr	0.011
5	0.002	Tr	-	Tr	-	0.001	-	Tr	Tr	0.003
6	0.001	0.001	-	Tr	-	0.001	-	Tr	Tr	0.003
7	0.002	0.002	-	0.006	-	0.013	0.006	0.003	0.001	0.084
8	0.003	0.001	-	0.002	-	0.002	-	Tr	Tr	0.023
9	0.002	0.001	-	-	-	Tr	-	-	-	-
10	0.001	0.001	-	0.001	-	0.001	-	-	-	0.005

[a] -, non-detected (< 0.0005 ppm); Tr, trace (<0.001 ppm - 0.0005 ppm>).

Appendix 8. Pesticide and PCB residues (µg/g wet weight) in adult female otter liver.

Sample #	HCB	αBHC	Lind	DDE	DDD	Chlordane Oxy	Chlordane Cis	Heptachlor Epoxide	Diel	PCB
1	0.001	Tr	-	0.001	-	0.001	-	Tr	-	0.011
2	0.001	Tr	-	0.001	-	0.001	-	Tr	-	0.007
3	0.003	Tr	-	0.001	-	0.001	-	Tr	-	0.008
4	0.002	Tr	-	0.001	-	0.001	-	Tr	-	Tr
5	0.002	Tr	-	-	-	0.001	-	Tr	-	0.015
6	0.003	Tr	-	0.001	-	0.001	-	Tr	-	0.007
7	0.004	Tr	-	0.001	-	0.002	-	Tr	-	0.006
8	0.003	Tr	-	0.003	-	0.002	-	Tr	-	0.058
9	0.004	Tr	-	0.023	0.005	0.003	0.001	0.001	-	0.020
10	0.003	Tr	-	0.002	-	0.001	-	0.001	-	0.015
11	0.005	0.002	-	0.004	-	0.001	Tr	0.001	-	0.009
12	0.003	Tr	-	0.001	-	Tr	-	0.001	-	0.003
13	0.001	-	-	Tr	-	-	-	-	-	0.005
14	0.002	-	-	0.001	-	Tr	-	Tr	-	0.010

[a] -, non-detected (< 0.0005 ppm); Tr, trace (<0.001 ppm - 0.0005 ppm>).

Appendix 9. Comparison of pesticide and PCB residues (µg/g wet weight)[a] in lipid samples (L) and pooled liver samples (LV) from male otters.

Sample #	Age	Tissue	HCB	αBHC	DDE	DDD	Chlordane Oxy	Chlordane Cis	Heptachlor Epoxide	Diel	PCB
1	SA	L1	0.097	0.038	-	-	-	-	-	-	1.370
		L2	NS	NS	NS	NS	NS	NS	NS	NS	NS
		LV	0.005	0.001	0.002	-	0.003	-	0.001	Tr	0.016
2	SA	L1	0.006	-	-	-	-	-	-	-	0.077
		L2	NS	NS	NS	-	NS	NS	NS	NS	NS
		LV	0.005	Tr	0.001	-	0.002	-	0.001	-	0.036
3	AD	L1	0.029	0.009	-	-	-	-	-	-	Tr
		L2	0.038	0.002	-	-	-	-	-	-	0.136
		LV	0.002	Tr	-	-	Tr	-	Tr	-	Tr
4	AD	L1	0.034	0.005	-	-	-	-	-	-	0.578
		L2	0.032	0.004	-	-	-	-	-	-	-
		LV	0.003	Tr	-	-	0.001	-	Tr	-	0.032
5	AD	L1	NS	NS	-	-	NS	-	NS	-	NS
		L2	0.024	-	-	-	-	-	-	-	0.403
		LV	0.001	Tr	-	-	0.001	-	Tr	-	0.007
6	AD	L1	0.012	-	-	-	-	-	-	-	0.762
		L2	0.024	-	-	-	-	-	-	-	0.121
		LV	0.002	Tr	-	-	0.001	-	0.001	-	0.026
7	AD	L1	0.021	-	-	-	-	-	-	-	0.984
		L2	0.025	0.017	-	-	-	-	-	-	0.098
		LV	0.002	Tr	Tr	-	0.001	-	Tr	-	0.021
8	AD	L1	0.029	0.030	0.020	-	-	-	-	-	1.245
		L2	0.032	0.047	0.040	-	-	-	-	-	0.390
		LV	0.002	Tr	0.001	-	0.001	-	Tr	-	0.046
9	AD	L1	0.036	0.018	0.004	-	-	-	-	-	1.190
		L2	0.016	0.015	-	-	-	-	-	-	0.348
		LV	0.002	Tr	Tr	-	0.001	-	Tr	-	0.024

[a] NS, no sample; -, non-detected (<0.0005 ppm); Tr, trace (<0.001 ppm - 0.0005 ppm>).

Appendix 10. Comparison of pesticide and PCB residues (µg/g wet weight)[a] in otter lipid samples (L) and pooled liver samples (LV) from female otters.

Sample #	Age	Tissue	HCB	αBHC	DDE	DDD	Chlordane Oxy	Chlordane Cis	Heptachlor Epoxide	Diel	PCB
1	SA	L1	0.020	0.040	-	-	-	-	-	-	-
		L2	0.028	0.035	-	-	-	-	-	-	-
		LV	0.002	0.001	-	-	-	-	-	-	-
2	SA	L1	0.015	0.028	-	-	-	-	-	-	-
		L2	0.027	0.004	0.027	-	-	-	-	-	0.340
		LV	0.001	0.001	0.001	-	0.001	-	-	-	0.005
3	SA	L1	0.068	0.060	0.005	-	-	-	-	-	0.309
		L2	NS	NS	NS	NS	NS	NS	NS	NS	NS
		LV	0.022	0.002	0.006	-	0.013	-	0.003	0.001	0.084
4	SA	L1	0.015	0.044	-	-	-	-	-	-	0.219
		L2	NS	NS	NS	NS	NS	NS	NS	NS	NS
		LV	0.001	0.001	Tr	-	0.001	-	Tr	Tr	0.003
5	SA	L1	0.013	0.018	-	-	-	-	-	-	0.145
		L2	0.078	0.041	0.032	-	-	-	-	-	0.677
		LV	0.004	0.001	0.003	-	0.001	-	-	-	0.031
6	SA	L1	0.016	0.020	-	-	-	-	-	-	0.480
		L2	0.037	0.023	0.158	-	-	-	-	-	2.340
		LV	0.003	0.001	0.002	-	0.002	-	Tr	Tr	0.023
7	AD	L1	0.018	0.030	-	-	-	-	-	-	0.034
		L2	NS	NS	NS	NS	NS	NS	NS	NS	NS
		LV	0.004	Tr	0.023	0.005	0.003	-	0.001	-	0.020
8	AD	L1	0.013	0.011	-	-	-	-	-	-	0.014
		L2	NS	NS	NS	NS	NS	NS	NS	NS	NS
		LV	0.003	Tr	0.001	-	Tr	-	0.001	-	0.003
9	AD	L1	0.060	0.013	0.008	-	-	-	-	-	0.115
		L2	0.017	0.014	-	-	-	-	-	-	0.290
		LV	0.003	Tr	0.001	-	0.001	-	Tr	-	0.008
10	AD	L1	0.064	0.054	0.056	-	-	-	-	-	0.331
		L2	NS	NS	NS	-	NS	NS	NS	-	NS
		LV	0.005	0.002	0.004	-	0.001	Tr	0.001	-	0.009
11	AD	L1	0.019	0.010	-	-	-	-	-	-	0.194
		L2	0.013	0.024	-	-	-	-	-	-	0.275
		LV	0.001	-	Tr	-	-	-	-	-	0.005

[a] NS, no sample; -, non-detected (<0.0005 ppm); Tr, trace (<0.001 ppm - 0.0005 ppm>).

Appendix 11. Method for perchlorination of PCB's.

1. Eluted samples were transferred to teflon-capped reaction vials with ~2 ml petroleum ether (PE).
2. 0.5 ml of 10^{-6} Aroclor 1254 or 1260 standard was pipetted into a reaction vial and 1 drop of keeper added.
3. Vials were immersed in a warm water bath, the PE was slowly evaporated to just dryness under an air stream, and 0.2 ml of $SbCl_5$ was added to all samples, standards and a blank vial.
4. Vials were capped and placed in a G.C. oven at 180°C overnight. After cooling at room temperature for 15 min., all vials were immersed in an ice bath for several min. and ~2 ml of 6N HCl was added.
5. Vials were swirled in the ice bath, ~2 ml of CH_2Cl_2 was added, and the vials were shaken and let stand.
6. After separation, the top CH_2Cl_2 layer was drawn off and placed into a test tube containing ~3-4 ml of saturated $NaHCO_3$ solution. The 2 ml CH_2Cl_2 extraction was repeated twice more, and the respective CH_2Cl_2 layers were added to the $NaHCO_3$.
7. After shaking and separation, the CH_2Cl_2 layer was transferred from the test tube to a 12 mm x 370 mm chromatography column filled with 12 cm of 5% deactivated florisil topped with anydrous Na_2SO_4.
8. The $NaHCO_3$ layer was extracted with a final 2 ml of CH_2Cl_2 and transferred to the chromatography column and the column was eluted with 20 ml of 25% CH_2Cl_2:PE(V:V).